CLAIMS

WE CLAIM:

1. A method for sequencing nucleic acids comprising:

(a) fragmenting large template nucleic acid molecules to generate a plurality of fragmented nucleic acids;

(b) delivering the fragmented nucleic acids into aqueous microreactors in a water-in-oil emulsion such that a plurality of aqueous microreactors comprise a single copy of a fragmented nucleic acid, a single bead capable of binding to the fragmented nucleic acid, and amplification reaction solution containing reagents necessary to perform nucleic acid amplification;

(c) amplifying the fragmented nucleic acids in the microreactors to form amplified copies of said nucleic acids and binding the amplified copies to beads in the microreactors;

(d) delivering the beads to an array of at least 10,000 reaction chambers on a planar surface, wherein a plurality of the reaction chambers comprise no more than a single bead; and

(e) performing a sequencing reaction simultaneously on a plurality of the reaction chambers.

2. The method of claim 1 wherein the reaction chambers have a center to center spacing of 20 to 100 μm.

3. The method of claim 1 wherein the fragmented nucleic acids are 30 – 500 bases.

4. The method of claim 1 wherein a plurality of the beads bind at least 10,000 amplified copies.

5. The method of claim 1 wherein step (c) is accomplished using polymerase chain reaction.

6. The method of claim 1 wherein the sequencing reaction is a pyrophosphate-based sequencing reaction.

7. The method of claim 1 wherein the sequencing reaction comprises the steps of:

(a) annealing an effective amount of a sequencing primer to the amplified copies of the nucleic acid and extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto a 3' end of said sequencing primer, a sequencing reaction byproduct; and

(b) identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid in a plurality of the reaction chambers.

8. The method of claim 1 wherein the sequencing reaction comprises the steps of:

(a) hybridizing two or more sequencing primers to one or a plurality of single strands of the nucleic acid molecule wherein all the primers except for one are reversibly blocked primers;

(b) incorporating at least one base onto the nucleic acid molecule by polymerase elongation from an unblocked primer;

(c) preventing further elongation of said unblocked primer;

(d) deblocking one of the reversibly blocked primers into an unblocked primer; and

(e) repeating steps (b) to (d) until at least one of the reversibly blocked primers are deblocked and used for determining a sequence.

9. The method of claim 1 wherein the reaction chambers are cavities formed by etching one end of a fiber optic bundle.

10. An array comprising a planar surface with a plurality of cavities thereon, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm and each cavity has a width in at least one dimension of between 20 μm and 70 μm, and wherein there are at least 10,000 reaction chambers.

11. The array of claim 10 wherein a plurality of reaction chambers contains at least 100,000 copies of a single species of single stranded nucleic acid template.

12. The array of claim 11 wherein the single stranded nucleic acid templates are immobilized on mobile solid supports disposed in the reaction chambers.

13. The array of claim 10 wherein the center to center spacing is between 40 to 60 μm.

14. The array of claim 10 wherein each cavity has a depth of between 20 μm and 60 μm.

15. An array comprising a planar top surface and a planar bottom surface wherein the planar top surface has at least 10,000 cavities thereon, each cavity forming an analyte reaction chamber, the planar bottom surface is optically conductive such that optical signals from the reaction chambers can be detected through the bottom planar surface, wherein the distance between the top surface and the bottom surface is no greater than 5 mm, wherein the reaction chambers have a center to center spacing of between 20 to 100μm and each chamber having a width in at least one dimension of between 20μm and 70μm.

16. The array of claim 15 wherein the distance between the top surface and the bottom surface is no greater than 2 mm.

17. The array of claim 10 or 15 wherein the number of cavities is greater than 50,000.

18. The array of claim 10 or 15 wherein the number of cavities is greater than 100,000.

19. The array of claim 10 or 15 wherein the shape of each reaction chamber is substantially hexagonal.

20. The array of claim 10 or 15 wherein each cavity has at least one irregular wall surface.

21. The array of claim 10 or 15 wherein the array is formed in a fused fiber optic bundle.

22. The array of claim 10 or 15 wherein each cavity has a smooth wall surface.

23. The array of claim 10 or 15 wherein the cavities are formed by etching one end of the fiber optic bundle.

24. The array of claim 10 or 15 wherein each cavity contains reagents for analyzing a nucleic acid or protein.

25. The array of claim 10 or 15 further comprising a second surface spaced apart from the planar array and in opposing contact therewith such that a flow chamber is formed over the array.

26. An array means for carrying out separate parallel common reactions in an aqueous environment, wherein the array means comprises a substrate comprising at least 10,000 discrete reaction chambers containing a starting material that is capable of reacting with a reagent, each of the reaction chambers being dimensioned such that when one or more fluids containing at least one reagent is delivered into each reaction chamber, the diffusion time for the reagent to diffuse out of the well exceeds the time required for the starting material to react with the reagent to form a product.

27. The array of claim 26 wherein each cavity contains reagents for analyzing a nucleic acid or protein.

28. The array of claim 26 further comprising a population of mobile solid supports disposed in the reaction chambers, each mobile solid support having one or more bioactive agents attached thereto.

29. The array of claim 26 wherein the cavities are formed in the substrate via etching, molding or micromaching.

30. The array of claim 17 wherein the substrate is a fiber optic bundle.

31. The array of claims 10, 15 or 26 wherein at least 5% to 20% of the reaction chambers contain at least one mobile solid support having at least one reagent immobilized thereon.

32. The array of claims 10, 15 or 26 wherein at least 20% to 60% of the reaction chambers have at least one mobile solid support having at least one reagent immobilized thereon.

33. The array of claims 10, 15 or 26 wherein at least 50% to 100% of the reaction chambers have at least one mobile solid support having at least one reagent immobilized thereon.

34. The array of claim 31 wherein the reagent immobilized on the mobile solid support is a polypeptide with sulfurylase activity.

35. The array of claims 31 wherein the reagent immobilized on the mobile solid support is a polypeptide with luciferase activity.

36. The array of claims 31 wherein the mobile solid support has both sulfurylase and luciferase immobilized.

37. The array of claim 31 wherein a plurality of reaction chambers contains at least 100,000 copies of a single species of single stranded nucleic acid template.

38. The array of claim 31 wherein the single stranded nucleic acid templates are immobilized on mobile solid supports disposed in the reaction chambers.

39. The array of claim 10, 15 or 26 wherein the nucleic acid is suitable for use in a pyrosequencing reaction.

40. A method for delivering a bioactive agent to an array, comprising dispersing over the array a plurality of mobile solid supports, each mobile solid support having at least one reagent immobilized thereon, wherein the reagent is suitable for use in a nucleic acid sequencing reaction, where the array comprises a planar surface with a plurality of reaction chambers disposed thereon, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm and each reaction chamber has a width in at least one dimension of between 20 μm and 70 μm.

41. An apparatus for simultaneously monitoring an array of reaction chambers for light indicating that a reaction is taking place at a particular site, the apparatus comprising:

(a) an array of reaction chambers formed from a planar substrate comprising a plurality of cavitated surfaces, each cavitated surface forming a reaction chamber adapted to contain analytes, and wherein the reaction chambers have a center to center spacing of

between 20 to 100 μm, each reaction chamber having a volume of between 10 to 150 pL, the array comprising more than 10,000 discrete reaction chambers;

(b) an optically sensitive device arranged so that in use the light from a particular reaction chamber will impinge upon a particular predetermined region of said optically sensitive device;

(c) means for determining the light level impinging upon each of said predetermined regions and

(d) means to record the variation of said light level with time for each of said reaction chamber.

42. An analytic sensor, comprising:

(a) an array formed from a first bundle of optical fibers with a plurality of cavitated surfaces at one end thereof, each cavitated surface forming a reaction chamber adapted to contain analytes, and wherein the reaction chambers have a center to center spacing of between 20 to 100 μm, a width of 20 to 70 μm, the array comprising more than 10,000 discrete reaction chambers;

(b) an enzymatic or fluorescent means for generating light in the reaction chambers;

(c) light detection means comprising a light capture means and a second fiber optic bundle for transmitting light to the light detecting means, the second fiber optic bundle being in optical contact with the array, such that light generated in an individual reaction chamber is captured by a separate fiber or groups of separate fibers of the second fiber optic bundle for transmission to the light capture means.

43. The sensor of claim 42 wherein said sensor is suitable for use in a biochemical assay.

44. The sensor of claim 42 wherein said sensor is suitable for use in a cell-based assay.

45. The sensor of claim 42 wherein the light capture means is a CCD camera.

46. The sensor of claim 42 wherein the reaction chambers contain one or more mobile solid supports with a bioactive agent immobilized thereon.

47. A method for carrying out separate parallel common reactions in an aqueous environment, comprising:

(a) delivering a fluid containing at least one reagent to an array, wherein the array comprises a substrate comprising at least 10,000 discrete reaction chambers, each reaction chamber adapted to contain analytes, and wherein the reaction chambers have a volume of between 10 to 150 pL and containing a starting material that is capable of reacting with the reagent, each of the reaction chambers being dimensioned such that when the fluid is delivered into each reaction chamber, the diffusion time for the reagent to diffuse out of the well exceeds the time required for the starting material to react with the reagent to form a product; and

(b) washing the fluid from the array in the time period (i) after the starting material has reacted with the reagent to form a product in each reaction chamber but (ii) before the reagent delivered to any one reaction chamber has diffused out of that reaction chamber into any other reaction chamber.

48. The method of claim 47 wherein the product formed in any one reaction chamber is independent of the product formed in any other reaction chamber, but is generated using one or more common reagents

49. The method of claim 47 wherein the starting material is a nucleic acid sequence and at least one reagent in the fluid is a nucleotide or nucleotide analog

50. The method of claim 47 wherein the fluid additionally comprises a polymerase capable of reacting the nucleic acid sequence and the nucleotide or nucleotide analog

51. The method of claim 47 additionally comprising repeating steps (a) and (b) sequentially.

52. A method for delivering nucleic acid sequencing enzymes to an array, said array having a planar surface with a plurality of cavities thereon, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm; the method comprising dispersing over the array a plurality of mobile solid supports having one or more nucleic acid

sequencing enzymes immobilized thereon, such that a plurality of the reaction chambers contain at least one mobile solid support.

53. The method of claim 52 wherein one of the nucleic acid sequencing enzymes is a polypeptide having sulfurylase activity, luciferase activity or both.

54. A method for delivering a plurality of nucleic acid templates to an array, said array having a planar surface with a plurality of cavities thereon, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm and the array having at least 10,000 reaction chambers; the method comprising dispersing over the array a plurality of mobile solid supports, each mobile solid support having no more than a single species of nucleic acid template immobilized thereon, the dispersion causing no more than one mobile solid support to be disposed within any one reaction chamber.

55. The method of claim 54 wherein the nucleic acid sequence is a single stranded nucleic acid.

56. The method of claim 54 wherein at least 100,000 copies of a single species of nucleic acid template are immobilized on a plurality of the mobile solid supports.

57. The method of claim 54 wherein each single species of nucleic acid template is amplified on a picotiter plate to produce at least 2,000,000 copies per well of said nucleic acid template after being disposed in the reaction chamber.

58. The method of claim 57 wherein the nucleic acid sequence is amplified using an amplification technology selected from the group consisting of polymerase chain reaction, ligase chain reaction and isothermal DNA amplification.

59. A method for sequencing a nucleic acid, the method comprising:

(a) providing a plurality of single-stranded nucleic acid templates disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm and the planar surface has at least 10,000 reaction chambers;

(b) performing a pyrophosphate based sequencing reaction simultaneously on all reaction chambers by annealing an effective amount of a sequencing primer to the nucleic acid templates and extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and

(c) identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid in each reaction chamber.

60. The method of claim 59, wherein the sequencing reaction byproduct is PPi and a coupled sulfurylase/luciferase reaction is used to generate light for detection.

61. The method of claim 60, wherein either or both of the sulfurylase and luciferase are immobilized on one or more mobile solid supports disposed at each reaction site.

62. A method of determining the base sequence of a plurality of nucleotides on an array, the method comprising:

(a) providing at least 10,000 DNA templates, each separately disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm, and a volume of between 10 to 150 pL; wherein

(b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates;

(c) detecting whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands in which incorporation of the nucleoside 5'-triphosphate precursor indicates that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and

(d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and

(e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors.

63. A method of identifying the base in a target position in a DNA sequence of template DNA, wherein:

(a) at least 10,000 separate DNA templates are separately disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm, said DNA being rendered single stranded either before or after being disposed in the reaction chambers,

(b) an extension primer is provided which hybridizes to said immobilized single-stranded DNA at a position immediately adjacent to said target position;

(c) said immobilized single-stranded DNA is subjected to a polymerase reaction in the presence of a predetermined deoxynucleotide or dideoxynucleotide, wherein if the predetermined deoxynucleotide or dideoxynucleotide is incorporated onto the 3' end of said sequencing primer then a sequencing reaction byproduct is formed; and

(d) identifying the sequencing reaction byproduct, thereby determining the nucleotide complementary to the base at said target position in each of the 10,000 DNA templates.

64. The method of claim 63 wherein in place of deoxy- or dideoxy adenosine triphosphate (ATP) a dATP or ddATP analogue is used which is capable of acting as a substrate for a polymerase but incapable of acting as a substrate for said PPi—detection enzyme.

65. An apparatus for analyzing a nucleic acid sequence, the apparatus comprising:

(a) a reagent delivery cuvette, wherein the cuvette includes an array comprising a planar surface with a plurality of cavities thereon, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 20 to 100 μm, and there are in excess of 10,000 reaction chambers, and wherein the reagent delivery cuvette contains reagents for use in a sequencing reaction;

(b) a reagent delivery means in communication with the reagent delivery cuvette;

(c) an imaging system in communication with the reagent delivery chamber; and

(d) a data collection system in communication with the imaging system.

66. An apparatus for determining the base sequence of a plurality of nucleotides on an array, the apparatus comprising:

(a) a reagent cuvette containing a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein there are in excess of 10,000 reaction chambers, each having a center to center spacing of between 20 to 100 μm and a volume of between 10 to 150 pL;

(b) reagent delivery means for simultaneously adding to each reaction chamber an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates;

(c) detection means for detecting in each reaction chamber whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands in which incorporation of the nucleoside 5'-triphosphate precursor indicates that the unpaired

nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and

(d) means for sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and

(e) data processing means for determining the base sequence of the unpaired nucleotide residues of the template simultaneously in each reaction chamber from the sequence of incorporation of said nucleoside precursors.

67. An apparatus for processing a plurality of analytes, the apparatus comprising:

(a) a flow chamber having disposed therein a substrate comprising at least 50,000 cavitated surfaces on a fiber optic bundle, each cavitated surface forming a reaction chamber adapted to contain analytes, and wherein the reaction chambers have a center to center spacing of between 20 to 100 μm and a diameter of 20 to 70 μm;

(b) fluid means for delivering processing reagents from one or more reservoirs to the flow chamber so that the analytes disposed in the reaction chambers are exposed to the reagents; and

(c) detection means for simultaneously detecting a sequence of optical signals from each of the reaction chambers, each optical signal of the sequence being indicative of an interaction between a processing reagent and the analyte disposed in the reaction chamber, wherein the detection means is in communication with the cavitated surfaces.

68. The apparatus of claim 67 wherein the detection means is a CCD camera.

69. The apparatus of claim 67 wherein the analyte is nucleic acid.

70. The apparatus of claim 67 wherein the analytes are immobilized on one or more mobile solid supports that are disposed in the reaction chamber.

71. The apparatus of claim 67 wherein the processing reagents are immobilized on one or more mobile solid supports.

72. A method for sequencing a nucleic acid, the method comprising:

(a) providing a plurality of single-stranded nucleic acid templates in an array having at least 50,000 discrete reaction sites;

(b) contacting the nucleic acid templates with reagents necessary to perform a pyrophosphate-based sequencing reaction coupled to light emission;

(c) detecting the light emitted from a plurality of reaction sites on respective portions of an optically sensitive device;

(d) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other reaction sites;

(e) determining the sequence of the nucleic acid templates based on light emission for each of said discrete reaction sites from the corresponding electrical signal.

73. The method of claim 1 further comprising the steps of:

(a) uniquely tagging fragmented nucleic acids from different biological sources libraries to create libraries of fragmented nucleic acids with different detectable sequence tags;

(b) sequencing said fragmented nucleic acids and detecting said detectable sequence tag from each said tagged nucleic acid fragment.

74. The method of claim 1 wherein the libraries are delivered individually or wherein the libraries are mixed and delivered simultaneously.

75. The method of claim 1 wherein said detectable sequence tag comprises an oligonucleotide of between 2 and 50 bases.

76. A method for sequencing nucleic acids comprising:

(a) fragmenting large template nucleic acid molecules to generate a plurality of fragmented nucleic acids;

(b) attaching one strand of a plurality of the fragmented nucleic acids individually to beads to generate single stranded nucleic acids attached individually to beads;

(c) delivering a population of the single stranded fragmented nucleic acids attached individually to beads to an array of at least 10,000 reaction chambers on a planar surface, wherein a plurality of the wells comprise no more than a one bead with on single stranded fragmented nucleic acid;

(d) performing a sequencing reaction simultaneously on a plurality of the reaction chambers.

77. The method of claim 76 wherein the reaction chambers have a center to center spacing of between 20 to 100 μm.

78. The method of claim 76 wherein the fragmented nucleic acids are between 30 – 500 bases.

79. The method of claim 76 wherein the fragmented nucleic acids are amplified in the reaction chambers prior to step (d).

80. The method of claim 76 wherein the amplifying step is accomplished using polymerase chain reaction.

81. The method of claim 76 wherein the sequencing reaction is a pyrophosphate-based sequencing reaction.

82. The method of claim 76 wherein the sequencing reaction comprises the steps of:

(f) annealing an effective amount of a sequencing primer to the single stranded fragmented nucleic acid templates and extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and

(g) identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid in a plurality of the reaction chambers.

83. The method of claim 76 wherein the sequencing reaction comprises the steps of:

(a) hybridizing two or more sequencing primers to one or a plurality of single strands of the nucleic acid molecule wherein all the primers except for one are reversibly blocked primers;

(b) incorporating at least one base onto the nucleic acid molecule by polymerase elongation from an unblocked primer;

(c) preventing further elongation of said unblocked primer;

(d) deblocking one of the reversibly blocked primers into an unblocked primer; and

(e) repeating steps (b) to (d) until at least one of the reversibly blocked primers are deblocked and used for determining a sequence.

84. The method of claim 76 wherein the reaction chambers are cavities formed by etching one end of a fiber optic bundle.